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Full Length Research Paper

16S rRNA phylogenetic analysis of actinomycetes isolated from Eastern Ghats and marine mangrove associated with antibacterial and anticancerous activities

Balasubramaniyam Nithya*, Ponnusamy Ponmurugan and Mangaladass Fredimoses

Department of Biotechnology, K. S. Rangasamy College of Technology, Tiruchengode - 637 215, Namakkal District, Tamil Nadu, India.

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Morphologically 238 different marine and terrestrial actinomycete strains were chosen by screening of the antagonistic activity using human pathogens and cancerous cell lines, for which 185 sediments/rhizosphere soil samples were collected from the different sites of Manakkudi estuary of south-west coastal regions and Yercaud hills of eastern Ghats of Tamil Nadu, India. The present study aimed at the evaluation of biologically diverse strains of actinomycetes from soil samples, for the production of wide spectrum of bioactive secondary metabolites. Most of the isolated strains belong to the genus namely *Streptomyces* (68%), followed by *Actinobacterium* (28%) and *Nocardiopsis* (4%). Among the different strains purified, about 10 strains exhibited high antagonistic activity. An attempt was made to study the bioactive compounds extracted from the Act2 strain isolated from marine mangrove sediments, which showed the highest antibacterial and anticancerous activities. The partially purified fifth active fraction was analyzed by Gas chromatography-mass spectrometry (GC-MS) and the possible closely related compounds were identified as Silane, Pyridine, 2,4,6-trimethyl, Amino malonic acid, 4-benzoxazin, Tris methyl and Cyclohexydimethoxy methyl compounds. Studies were conducted to extract the genomic DNA from the selected strains, amplified through polymerase chain reaction (PCR) and subjected to 16S rRNA sequence analysis. Subsequently, phylogenetic tree was constructed using suitable bioinformatics tools to identify the similarity which showed 97% similarity between strains. Moreover, all the selected strains of actinomycetes were subjected to study the protein and plasmid DNA expression profiles which showed prominent bands with genetically excellent relationship between strains in the profiles.

Key words: Marine mangrove, Yercaud hills, actinomycetes, antibacterial activity, anticancerous activity, bioactive compounds, 16S rRNA sequence, phylogenetic tree.

INTRODUCTION

Actinomycetes represent a ubiquitous group of microbes widely distributed in natural ecosystems around the world, and subjected to produce bioactive principles. Marine mangroves and eastern Ghats of Indian eco-systems facilitate the growth of both beneficial and harmful diverse microorganisms. The higher productivity in mangrove swamps supports a large number of micro-organisms (Alongi, 2002). Marine microorganisms are increasingly becoming an important source for the search of industrially important molecules on a large scale level (Dharmaraj, 2010). Today, both academic and industrial interest in marine microorganisms is on the rise because unique and biologically active metabolites have been reported from marine organisms (Imada et al., 2007; Zhang et al., 2005). In contrasts, the eastern Ghats are a

*Corresponding author. E-mail: nithu2006@gmail.com. Tel: 04288-274741, 91-94422-93253. Fax: 04288-274745.

discontinuous range of mountains along India's eastern coast, wherein, different kinds of exudates at the plant rhizosphere region provide substrate to the soil microorganisms (Ponmurugan and Nithya, 2008). The microbial natural products were screened from the plant rhizosphere region for the development of new therapeutic agents (Kurtboke and Wildman, 1998).

Actinomycetes have high guanosine+cytosine [(G) + (C)] content in the genome, and they are free living microorganism. They can produce an array of secondary metabolites, many of which have antibacterial/antifungal or antitumor properties (Sanglier et al., 1993). They are considered highly valuable, as they produce various antibiotics and other therapeutically useful compounds with diverse biological activities (Goodfellow et al., 1984). The vast majority of these metabolites (70%) have been isolated from actinomycetes, with the remaining 20% from fungi, 7% from *Bacillus* and 1 to 2% from *Pseudomonas* (Ramesh and Mathivanan, 2009). Numerous chemical compounds, including antibiotics, enzymes, vitamins, growth hormones and antitumor agents are widely obtained from *Streptomycetes* (Saadoun and Gharaibeh, 2002). Several species from the *Streptomyces* genus produce bioactive molecules, including enzymatic inhibitors with antibiotic activity, and many of them are commercially valuable enzymes such as lipases, cellulases and proteases (Ravel et al., 2000).

In recent years, intensive research had focused on identification of new natural antitumor agents derived from various plants, marine organisms, animals and microorganisms. The distinct class of secondary metabolites obtained from actinomycete bacteria has enough potential, as well as scope for anticancerous activities. Cancer still represents one of the most serious human health problems despite the great understanding of its biology and pharmacology. The usual therapeutics methods for cancer treatment are surgery, radiotherapy, immunotherapy and chemotherapy (Cocco et al., 2003). An analysis of the number of chemotherapeutic drugs and their sources indicated that over 60% of approved drugs are derived from natural compounds (Newman and Cragg, 2004), and many of them have been extracted from actinomycetes. Similarly, infectious diseases of human beings are leading health problems with high morbidity and mortality in the developing countries like India (Krishnakumari et al., 2006). The development of resistance to multiple drugs is a major problem in the treatment of infectious diseases caused by pathogenic microorganisms. Actinomycetes are potential source of many bioactive compounds (Xu et al., 2005) which have diverse clinical effects and important applications in medicine for treating various human diseases and disorders (Watve et al., 2001). Actinomycete groups have been detected and characterized by their 16S rRNA sequences in cases where cultivation has proved unsuccessful (Rheims et al., 1996). The diversity analysis by ribotyping with 16S rRNA phylogenetic marker showed that a group of high GC rich Gram-positive bacteria (actinomycetes) are dominant in marine sediments (Urakawa et al., 1999) for intending biomedical applications. 16S rRNA sequencing method is being considered as the most important powerful tool to screen the isolated actinomycetes at molecular level (Wellington et al., 1994).

Manakkudi estuary of south-west coastal regions and Yercaud hills of eastern Ghats of Tamil Nadu, southern India is not explored for microbial metabolites for antibacterial and anticancerous activities. Hence, there is an immense possibility to identify industrially important marine and terrestrial associated actinomycetes in these two areas, to discover novel bioactive compounds for the management of infectious and non-infectious diseases in human beings.

MATERIALS AND METHODS

Study area

For the present study, sediment samples were collected from Manakkudi estuary (Latitude: 8° 05' E; Longitude: 77° 46' E; Altitude: 117 m mean sea level) situated in the south-west coastal regions of Tamil Nadu state, southern India. Similarly, rhizosphere soil samples were collected from the medicinal plants at Yercaud hills (Latitude: 11° 76' E; Longitude: 78° 23' E; Altitude: 1515 m mean sea level) of eastern Ghats of Tamil Nadu for the present study (Figure 1). These samples were subjected to analysis for various soil parameters, and to isolate morphologically different actinomycetes, using Starch casein nitrate agar medium. The isolated actinomycetes were characterized by morphological and biochemical traits. The results were published earlier by Ponmurugan and Nithya (2008) and Ravikumar et al. (2011). A total of 238 different marine and terrestrial actinomycete strains were obtained from the above samples. The strains such as ACT1 to ACT5 and NMK1, Fvkcu1, Ukkvf1, KSR3 and Mosae 1 strains were obtained from the site of Manakkudi estuary and Yercaud hills, respectively.

Extraction of bioactive secondary metabolites

Antibiotic production medium (g/l: 25 starch, 10 glucose, 2 yeast extract, 3 calcium carbonate and one ml of trace solution containing $ZnSO_4$, $MnCl_2$, $CuSO_4$ and $FeSO_4$, pH 7.5) was used for extraction of bioactive secondary metabolites from actinomycete cultures. Based on the *in vitro* performance like growth pattern and colour with culture characteristics and antibacterial/antitumor activities described by Goodfellow et al. (1984), the pure culture of ACT1 strain was selected for the study. It was inoculated into 250 ml of seed medium in 3 L capacity of fermentor (Bioconsole, USA) vessel for 25 days. The culture filtrate was centrifuged at 11000 rpm to get a clear solution, and filter sterilized. The final filtrate was mixed with ethyl acetate (2:1) in a separating funnel to extract the bioactive compounds. After removing the lower aqueous phase, the upper solvent phase was concentrated to obtain crude extract. This crude extract was used for further screening (Bauer et al., 1996).

Screening of marine and terrestrial actinomycetes

The antibacterial activity of bioactive compounds extracted from marine and terrestrial actinomycetes was tested against a few human bacterial pathogens using Muller-Hinton agar plates by following well-diffusion method, in which the zone of inhibition was calculated (Dhingra and Sinclair, 1995). The human bacterial pathogens covering Gram-negative bacteria, such as *Escherichia coli* MTCC 1687, *Pseudomonas aeruginosa* MTCC 2642, *Salmonella enteritidis* MTCC 453 and *Shigella dysenteriae* MTCC 2405 and Gram-positive bacteria such as

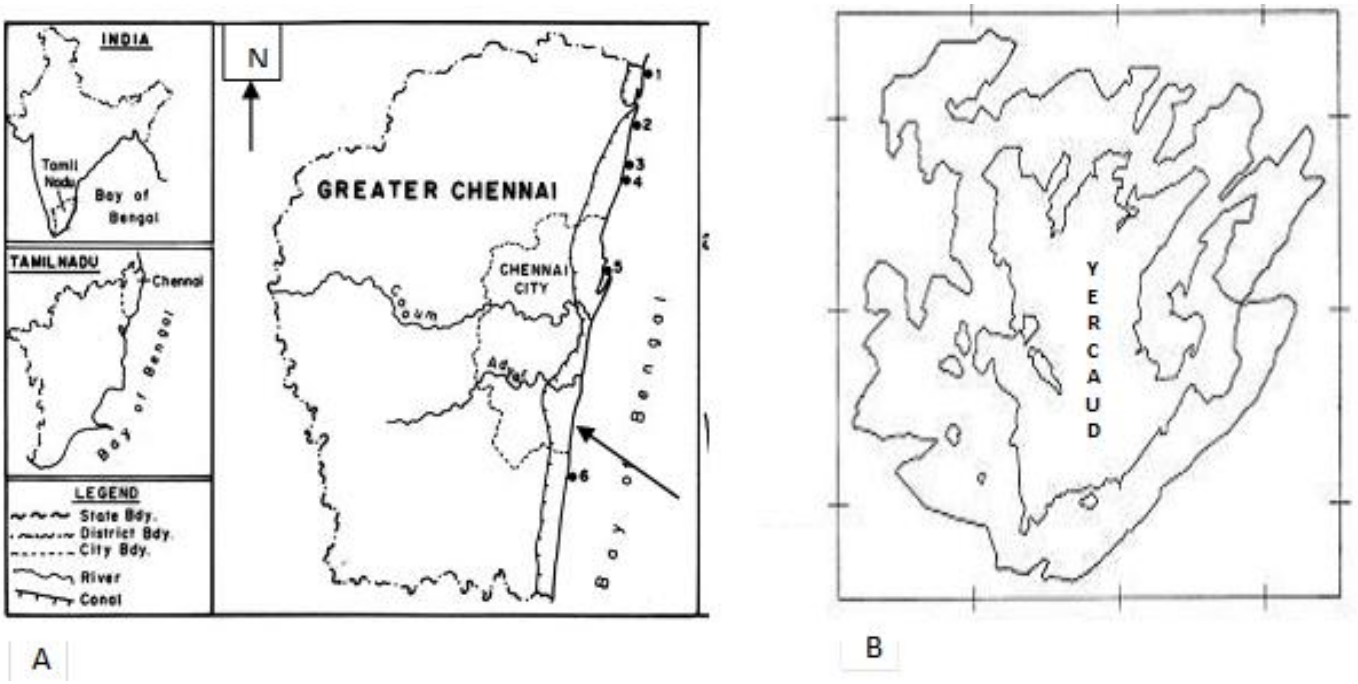

Figure 1. Sampling locations of Manakkudi estuary of south-west coastal regions (A) and Yercaud hills of eastern Ghats, (B) of Tamil Nadu, India. Arrow indicates the sampling site at Manakkudi estuary.

Staphylococcus aureus MTCC 7405, *Streptococcus pyogenes* MTCC 442, *Clostridium botulinum* KSRCT 157 and *Bacillus subtilis* MTCC 2387 were selected for the study. Similarly, the anticancerous activity of the same bioactive compounds was evaluated using human cancerous cell lines such as SKBR-3, MCF-7 and MDA-MB-231. The cells were grown in minimal essential medium with Eagle's salts containing 10% fetal bovine serum, and maintained in 5% CO_2 at 37°C in a CO_2 incubator (Balaraman and Prabakaran, 2007). The cell suspensions at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates, and incubated under controlled condition. The bioactive secondary metabolites solution (DMSO), at 50 µl of 80% Trichloroacetic acid (TCA) was added for testing purpose (Fredimoses, 2010). The absorbance of the treated samples was measured at 540 nm, by using microtiter plate reader (Elisa, India).

Purification and identification of bioactive compounds

The crude solvent extract was subjected to Silica gel chromatography (22 × 5cm, Silica gel 60, Merck) and eluted with gradient solvent system consisting of ethyl acetate + hexane. Eluents collected during column chromatography were concentrated. The partially purified fifth active fraction was analyzed by gas chromatography-mass spectrometry (GC-MS) equipped with a fused silica capillary column (CW-amine 60 × 0.25 mm I.D., Film thickness 0.5 µm). Column condition was programmed as column oven temperature at 150°C (4 min) -4°C/min, 250°C for injection port and 280°C for detector. The peaks of the components were subjected to mass spectral analysis on NIST MS Search (Version 2.0) (Roy et al., 2006).

Separation of proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

All the selected strains of actinomycetes were grown on yeast-malt extract broth (g/l: 3 yeast extract, 5 bacto-peptone, 3 malt extract, 10 glucose, 30 sucrose, 5 glycine and 2 ml of 2.5 M $MgCl_2.6H_2O$ solution) at 28°C under shaking at 200 rpm for 20 days. Actinomycetes-hyphae were harvested and ground with phosphate buffer (pH 7.0). These samples were centrifuged at 6000 rpm for 5 min, in which supernatants were discarded. The pellets were resuspended in 40 µl TE buffer to which 6 µl of SDS and 2 µl of loading dye were added. The samples were heat denatured at 95°C for 5 min and then loaded on the SDS-PAGE gel. After electrophoresis, the gel was carefully transferred to Coomassie Brilliant Blue staining solution [0.5 g of Coomassie Brilliant Blue R250 (Sigma, Germany) in 90 ml methanol (1:1 v/v) and 10 ml glacial acetic acid], followed by destaining solution (30% methanol and 10% acetic acid) on a rocker. Finally, the results were documented and photographed using a Gel Documentation System (Alpha Digitoc, New York, US) (Laemmli, 1970).

Isolation of plasmid DNA from actinomycetes

All the selected strains of actinomycetes were grown on the above yeast-malt extract broth at 28°C under shaking, at 200 rpm for 20 days. Actinomycetes-hyphae were harvested by filtration, suspended in 1 ml of TE buffer, and subsequently lysed by using freshly prepared lysis buffer. It was incubated for 1 h at 55°C, and extracted with 6 ml of phenol-chloroform (1:1 V/V). After centrifugation, the supernatant was subjected to agarose gel electrophoresis using 0.7% agarose gels. Gels were viewed under an ultraviolet (UV) Transilluminator (Bangalore Genei, India) and then photographed using a Gel Documentation system (Kieser, 1984).

Isolation of genomic DNA from actinomycete cultures

The actinomycete cultures were grown in 25 ml of Trypticase soya broth (g/l: 17 casein enzyme hydrolysate, 3 papaic digest of soya bean meal, 5 NaCl, 2 K_2HPO_4 and 2.5 dextrose). The cells were centrifuged at 10000 rpm, and the pellet was resuspended in 1 ml of TE buffer containing 20 mg of lysozyme mL^{-1} and 20 mg of RNAase A mL^{-1}. It was incubated at 37°C for 1 h, after incubation, 250 µl of 0.5 M EDTA, 250 µl of TE containing 5 mg of proteinase K mL^{-1} and 100 µl of 10% SDS were added. 200 µl of Cetyltrimethyl ammonium bromide (CTAB) solution

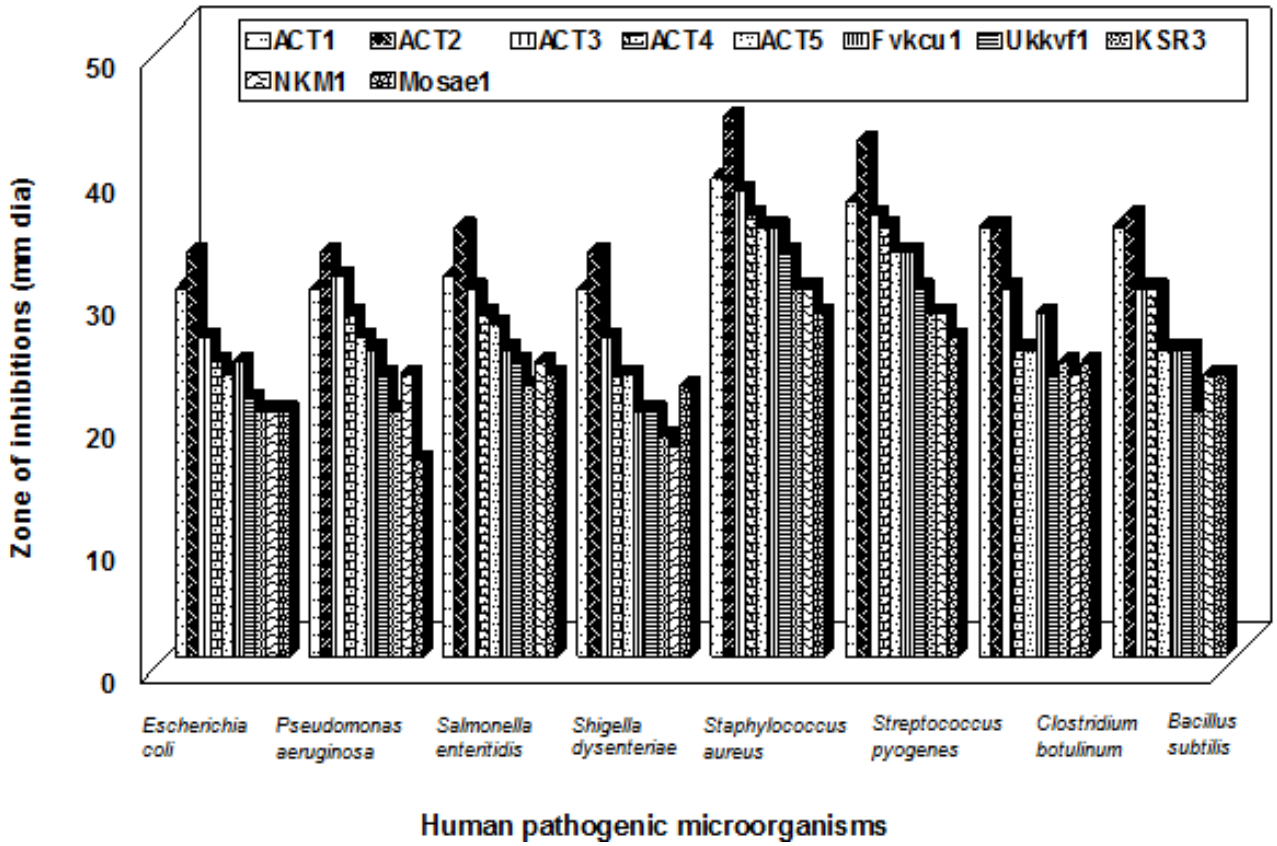

Figure 2. Antibacterial activity of different marine and terrestrial actinomycetes strains against human pathogens.

(10% CTAB plus 0.7 M NaCl) was added and heated in a 65°C water bath for 10 min. The aqueous phase was taken, and the DNA was precipitated with an equal volume of isopropanol (Sambrook et al., 2007).

16S rRNA gene amplification and DNA sequencing

The 16S rRNA gene was amplified from genomic DNA obtained from actinomycete cultures by PCR with forward primer-F243 (5'-GGATGAGCCCGCGGCCTA-3') and reverse primer R513GC (5'-CGGCCGCGGCTGCTGGCACGTA-3'). The reaction mixture contained 50 ng of DNA, Ex Taq PCR buffer, 1.5 mM $MgCl_2$, 10 mM deoxynucleoside triphosphate mixture, 50 pmol of each primer and 0.5 U of Ex Taq polymerase chain reaction (PCR). Conditions consisted of an initial denaturation at 94°C for 5 min; 30 cycles at 94°C for 1 min, annealing at 63°C for 1 min and 72°C for 1 min; and final 5 min extension at 72°C. The amplification products were examined by agarose gel electrophoresis and purified by using a QIA quick PCR clean up kit (Fredimoses, 2010). The complete 16S rRNA gene was sequenced by using an ABI 377 automated DNA sequencer.

Phylogenetic analysis

Nucleotide sequences were compared to those in the Gene Bank database with the Basic local alignment search tool (BLAST) algorithm to identify known closely related sequences. Sequences were analyzed with Chromo software, and thereby trees were generated by the neighbor-joining algorithm implemented in phydit (Saitou and Nei, 1987). The assemblage of 16S rDNA gene sequences in each library was analyzed by rarefaction analysis using EcoSim (Gotelli and Entsminger, 2006) to assess the extent to which the diversity of microbial communities was represented by the library at the class and species level. The number of species in each clone library was determined by comparing closely related sequences using bl2seq (http://www.ncbi.nlm.nih.gov/blast/ bl2seq/wblast2.cgi) according to Tatusova and Madden (1999). 16S rDNA sequences exhibiting a percentage of similarity of 97% or lower were considered for species authentication (Devereux et al., 1990).

RESULTS AND DISCUSSION

Actinomycetes constantly hold a special significance in the research area during the last five decades as the members of this group, especially; *Streptomycetes* are known to produce an array of bioactive compounds with diverse biological properties (Williams, 2009). The discovery of new bioactive compounds is a never ending process to meet the everlasting demand for novel drugs and other biomolecules with antimicrobial and therapeutic properties, in order to combat bacterial pathogens in human beings (Ramesh and Mathivanan, 2009). However, it is more important to identify newer or rare actinomycete species from marine and terrestrial sources because they are the pivotal source of potent molecules. Marine and alkaline soil environments are a noteworthy feature to identify novel organisms with various biological properties due to alkaline nature of the habitat. Around 108 terrestrial actinomycete strains were

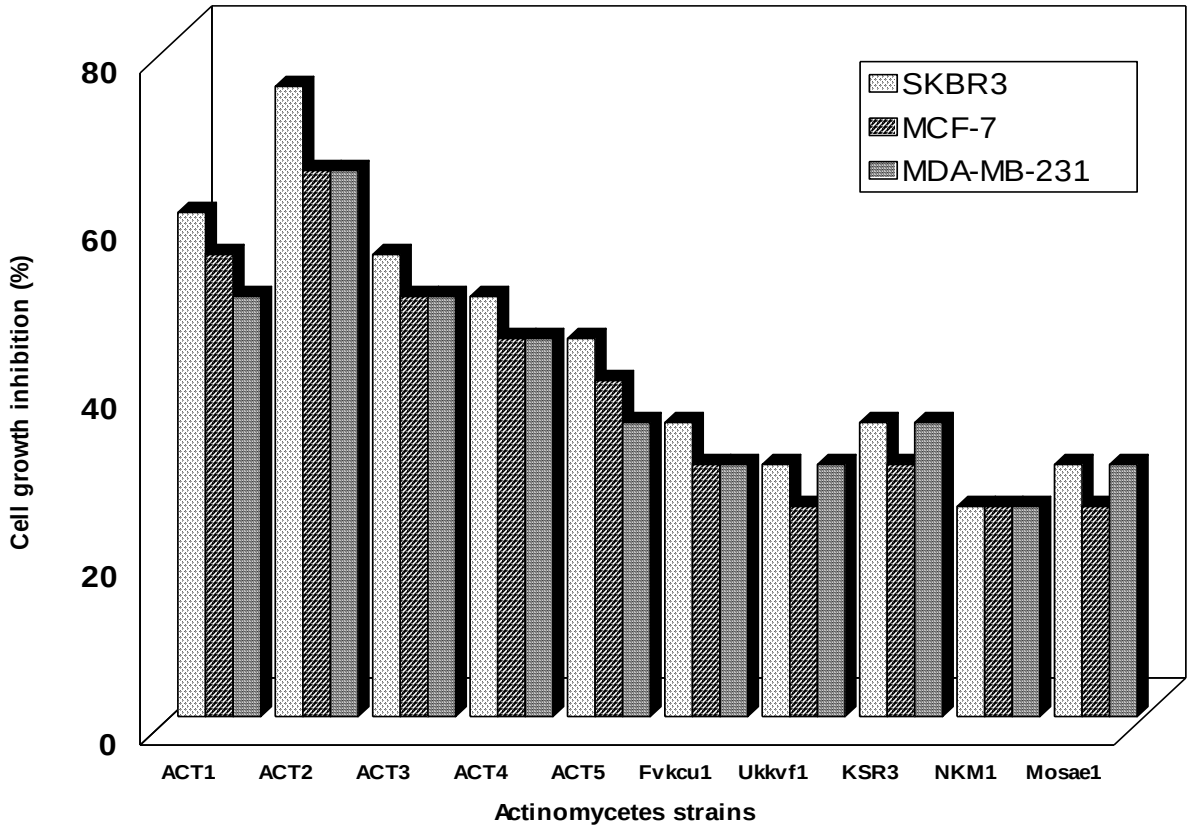

Figure 3. Anticancerous activity of different marine and terrestrial actinomycetes strains against human cancerous cell lines.

picked up from soil samples collected from Yercaud hills, and 130 marine actinomycete strains were obtained from Manakkudi regions. The degree of antibacterial activity varied greatly among the actinomycete strains as shown in Figure 2. The overall results on antibacterial activity showed that actinomycete strains obtained from Manakkudi mangrove regions were found to be superior to the strains picked up from the site of Yercaud hills. Among the two groups of bacteria tested, Gram-positive bacteria were superior to Gram-negative bacteria in terms of the maximum zone of inhibition by all the 10 strains of actinomycetes; of which, Act2 strain exhibited the excellent antibacterial activity against all the pathogenic microorganisms tested, followed by Act1 and Act3 strains. The maximum zone of inhibition (47 mm diameter) was noticed by the strain Act2 against *S. aureus* followed by *S. pyogenes*. Several researchers had already reported similar antimicrobial activity of actinomycetes against various human pathogens (Krishnakumari et al., 2006; Thangapandian et al., 2007). According to Sattler et al. (1998), most of the metabolites extracted from actinomycetes inhibit the growth of Gram-positive bacteria, but are ineffective against Gram-negative bacteria due to alternation of peptidoglycan and its building blocks of amino acids in the cell wall (Fredimoses, 2010). Saadoun and Gharaibeh (2002) obtained 90 different *Streptomyces* isolates, of which, 54% exhibited remarkable antibacterial activity against *B. subtilis, S. aureus, E. coli, Klebsiella* sp. and *Shigella* sp. Similarly, Deshmukh and Sridhar (2002) isolated several actinomycetes from freshwater coastal stream, of which, four isolates inhibited the growth of *B. subtilis* and *E. coli*.

The results on the evaluation of bioactive compounds using cancerous cell lines indicate that the percentage of growth inhibition was maximum in SKBR3, followed by MCF-7 and MDA-MB-231 cell lines (Figure 3). Among the different strains tested, Act2 strain exhibited the excellent anticancerous activity against the entire cancerous cell lines used, followed by Act1 and Act3 strains. These results coincide with the observation of antibacterial activity. The anticancerous activity of secondary metabolites obtained from actinomycetes group has been studied by several investigators (Shipley et al., 2009; She et al., 2006). The growth inhibition of cancer cells occurred due to cytotoxic effects, cell wall lysis, tissue damage and apoptosis nature of secondary metabolites. Shipley et al. (2009) carried out a study to demonstrate the antitumor activity of secondary metabolites extracted from *Streptomyces* species, which revealed that various arrays of secondary metabolites inhibited the cancer cells in varying degrees. The cell death may be a consequence of host cell infection or due to the toxic products

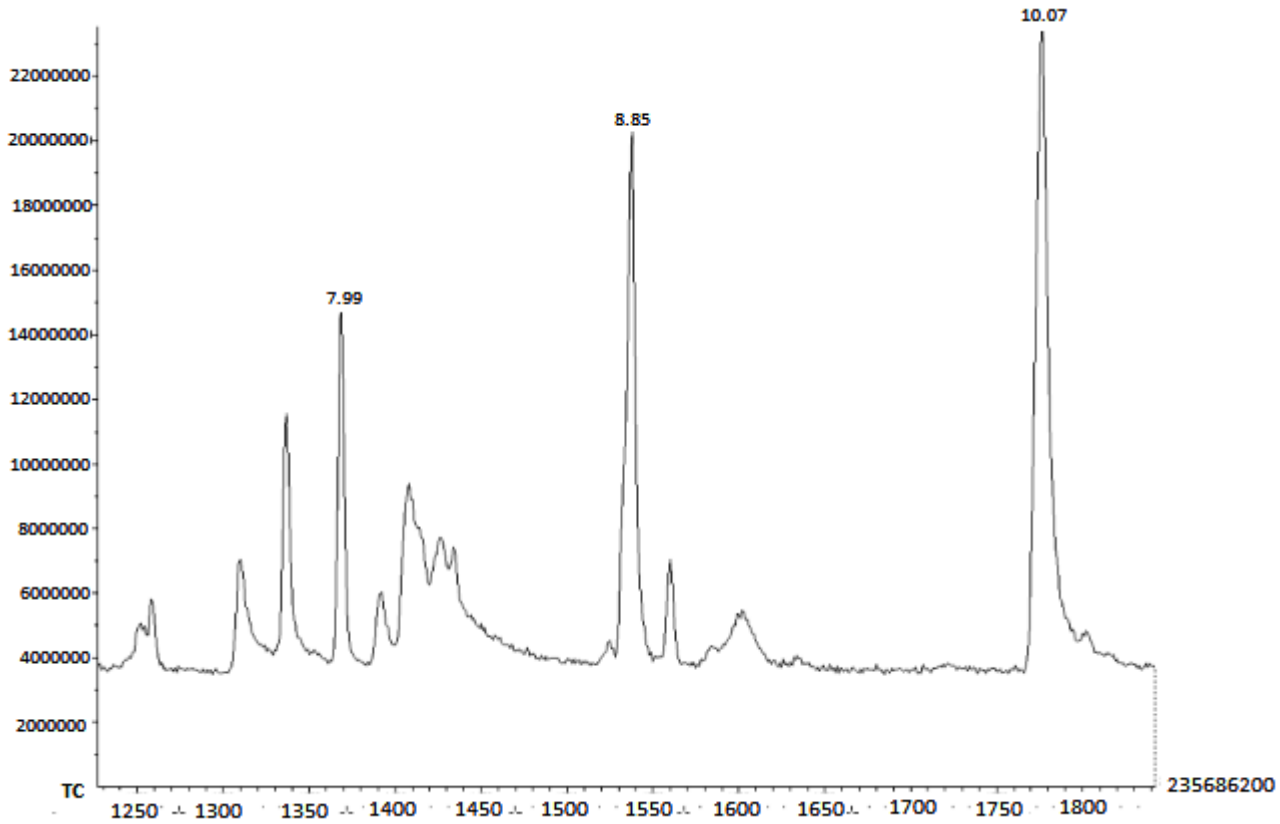

Figure 4. GC-MS analysis of culture broth of marine and terrestrial actinomycetes strain extracted by using ethyl acetate.

absorbed by the cell lines. Moreover, the most widely accepted theory is that antibiotics are used to compete with living organisms and cells in nutrient depleting environment (Demain and Fang, 1995). The most promising strain of Act2, crude extract alone, was subjected to the column chromatography to get different fractions. The partially purified fifth fraction of the bioactive compounds was further analyzed by GC-MS instrument. There were three prominent peaks with retention time of 7.99 (C1), 8.85 (C2) and 10.07 (C3) min, which suggested 32.11, 79.09 and 136.19 of molecular weight, respectively. By using available library data, C1, C2 and C3 were determined as silane and pyridine (7.99 min retention time), 2,4,6-trimethyl, amino malonic acid and 4-benzoxazin (8.85) and Tris methyl and cyclohexydimethoxy methyl (10.07) compounds, respectively (Figure 4). It has been reported that actinomycetes are known to produce an array of bioactive secondary metabolites which are anthracene, quinone and qnthraquinone derivatives in general (Goto et al., 1998). These compounds have different diversified applications in biomedical research (Saadoun and Gharaibeh, 2002). All the ten selected strains of actinomycetes exhibited prominent protein pattern and all of them were closely related (Figure 5). All the strains had more than 15 common protein bands with one prominent band lying at the molecular weight of 45 Kda. Moreover, a number of bands and their prevailing nature were found to be slightly higher in few strains, which were obtained from mangrove regions than the other strains isolated from the site of Yercaud hills. Protein pattern is a widely used technique for the identification and differentiation between the strains through polyacrylamide gel electrophoresis. The difference in banding pattern between strains provides a good measure of changing gene functions, especially genes encode for proteins and differential gene action so as to achieve specific protein proportions. The protein expression profile may be further useful in chemotaxonomic studies upon secondary metabolite production in actinomycetes (Wlck and Liu, 1997; Ponmurugan et al., 2010).

Similarly, there was a close relationship between strains of actinomycetes obtained from mangrove regions and Yercaud hills in terms of plasmid DNA profile (Figure 6). Lower molecular weight CCC-DNA was detected from all the strains of actinomycetes, with a common unique prominent band at the molecular weight of 15.2 MDa. The strains such as ACT1, ACT2, Ukkvf1, KSR3, NKM1 and Mosae1 exhibited two prominent plasmids in the profile. The molecular weight of plasmids of all the strains was

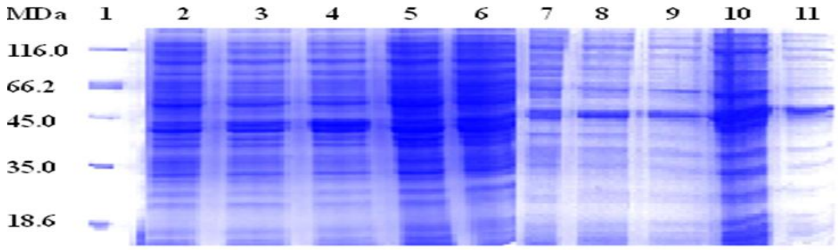

Figure 5. Protein expression profile of different marine and terrestrial actinomycete strains. Lane 1, Marker DNA; Lane 2 to 11, different strains of actinomycetes (2, ACT1; 3, ACT2; 4, ACT3; 5, ACT4; 6, ACT5; 7, Fvkcu1; 8, Ukkvf1; 9, KSR3; 10, NKM1; 11, Mosae1).

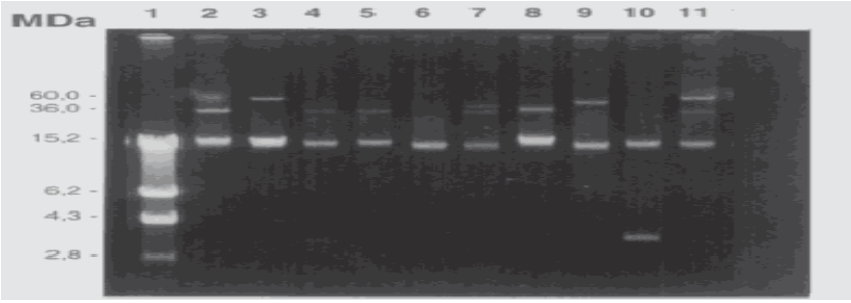

Figure 6. Plasmid DNA profile of different marine and terrestrial actinomycetes strains. Lane 1, Marker DNA; Lane 2 to 11, different strains of actinomycetes (2, ACT1; 3, ACT2; 4, ACT3; 5, ACT4; 6, ACT5; 7, Fvkcu1; 8, Ukkvf1; 9, KSR3; 10, NKM1; 11, Mosae1).

Table 1. Preliminary classification of cultivable actinomycete strains and genebank accession numbers of the 16S rRNA gene sequences of the 10 strains.

Strain code	Details of similar species	Accession number	Identity (%)
Act-1	*Actinobacterium* species - ACT1	GQ478246	98
Act-2	*Streptomyces* species - ACT2	GQ478247	100
Act-3	*Actinobacterium* species - ACT3	GQ478248	98
Act-4	*Streptomyces* species - ACT4	GQ478249	100
Act-5	*Actinobacterium* species - ACT5	GQ478250	98
Act-6	*Streptomyces* species - Fvkcu1	GU985264	100
Act-7	*Streptomyces* species - Ukkvf1	GU985265	100
Act-8	*Streptomyces* species - KSR3	GU985265	100
Act-9	*Streptomyces* species - NKM1	HM125709	100
Act-10	*Nocardiopsis* species - Mosae1	GU353189	98

ranged between 60 and 15.2 MDa, except NKM1 strain. From the study, it is inferred that all the ten strains were closely related in terms of protein and plasmid DNA expression profiles. Saadoun et al. (1998) observed that only CCC-DNA is not linear DNA in their samples containing the genus of *Streptomyces*. They were further suggestions that antibiotic production in these strains is likely to be chromosomally encoded. Four different extraction methods of small plasmid DNA from antibiotic-producing *Streptomyces* isolates and from the positive control *S. lividans*, containing the pIJ702 plasmid, were standardized. Among these, only one procedure allowed the detection of plasmid DNA from the positive control very effectively, and that was the Kieser (1984) method. This method is widely used now for the extraction of plasmid DNA from actinomycetes (Saadoun et al., 1998) and this is in agreement with our results.

The 16S rRNA sequence analysis of all the 10 strains as representatives of the diversity isolated and partially sequenced is summarized in the Table 1. A BLAST

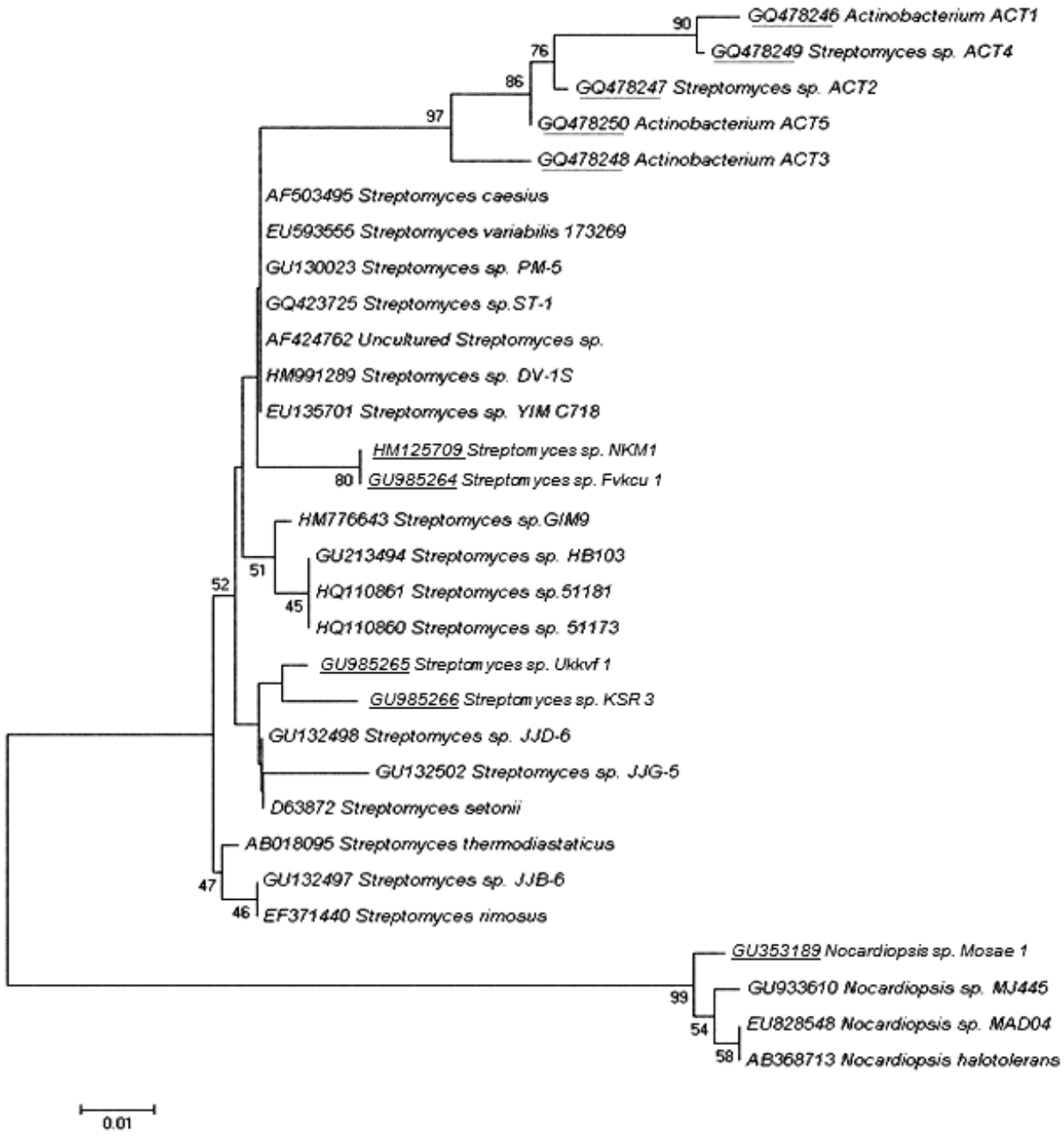

Figure 7. Construction of phylogenetic tree for marine and terrestrial actinomycete strains**.** The evolutionary history was inferred using the Neighbor-Joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was shown next to the branches (frequencies below 40% disregarded). The GenBank accession number together with the culture collection number for each strain was listed; the accession numbers underlined are our own strains.

analysis carried out through blastn search through GenBank (http://www.ncbi.nlm.nih.gov) revealed that over six strains (ACT4, ACT2, NKM1, Fvkcu1, Ukkvf1 and KSR3) out of 10 are the members of the genus *Streptomyces*. The strains such as ACT1, ACT3 and ACT5 belong to the member of the genus *Actinobacterium* and Mosae1 strain is the member of *Nocardiopsis.* The construction of phylogenetic tree revealed 16S rRNA gene sequence identity of 96% similarity to uncultured *Actinobacterium* and 100% similarity to *Streptomyces* were noticed (Figure 7).

Molecular tools have a great potential to assist in isolating

yet-uncultured bacteria with known rRNA sequences for further investigations (Teske et al., 1996). One of these molecular tools is the PCR amplification of variable regions of the genes encoding 16S rDNA, by use of primers homologous to conserved regions of the gene. However, there are numerous reports based on 16S rRNA gene sequence data, which suggest that more than 99% of microorganisms in natural habitats are uncultured or uncultivable one (Whitman et al., 1998). In this present investigation, 16S rRNA amplified products were sequenced, which revealed that six strains belong to the members of the genus *Streptomyces,* which was the dominant actinobacterial genus. Ellaiah and Reddy (1987) isolated actinomycetes from natural habitats and found that the most common genus was *Streptomyces*. Similar predominance of *Streptomyces* was also reported from marine sediments and alkaline environments (Hans-Peter et al., 2005). The results of the present study are in agreement with earlier findings which states that *Streptomyces* species are mainly found in shelf and shallow areas when compared to other genera of actinomycetes (Thorne and Alder, 2002).

Conclusion

In this study, the selected strains of actinomycetes were found to be of potential candidate for antibacterial and anticancerous activities. The search for wide spectrum of antimicrobial metabolites, especially from actinomycetes, requires a large number of isolates in order to discover a novel compound of pharmaceutical interest. Thus, it can be concluded from the results of the present investigation that the marine actinomycetes from Manakkudi mangrove environment are a potential source of novel antibiotics in biomedical applications. Moreover, it is important to understand the marine-derived actinomycetes in ecological terms and also as a resource for bioprospecting.

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